IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	F.C. Prats
Art Unit	:	1651
Applicant	:	Bruce Joseph Roser
Serial No.	:	08/875,796
Filed	:	October 30, 1998
For	:	Dried Blood Factor Compositions Comprising Trehalose

DECLARATION OF ALAN P. MACKENZIE UNDER 37 CFR §1.132

I, hereby declare that:

I have the following educational background:

Univ. of London, England	B.Sc.	1951	General Science
Univ. of London, England	B.Sc.	1952	Chemistry
Univ. of London, England	Ph.D.	1957	Chemistry

Honors: Both B.Sc. degrees were taken with First Class Honours.

I have held the following Research and Professional Appointments:

Research Associate Professor, Center for Bioengineering, School of Medicine, University of Washington, Seattle, Washington.

Associate Professor, Center for Bioengineering, School of Medicine, University of Washington, Seattle, Washington.

Research Associate, High Voltage Electron Microscope Laboratory, University of Wisconsin, Madison, Wisconsin.

Associate Director, Cryobiology Research Institute, Madison, Wisconsin.

Associate Director of Research, American Foundation for Biological Research, Madison, Wisconsin.

Research Associate, American Foundation for Biological Research, Madison, Wisconsin.

Staff Scientist, Glaxo Research, Glaxo Laboratories, Ltd., Greenford, Middlesex, England.

The following are representative of my technical publications:

MacKenzie, A.P. and Luyet, B.J.: A collodion sandwich-film technique for the study of the growth of ice in very thin layers of aqueous solutions. In "Electron Microscopy, Vol. 2" (Proceedings of the Fifth International Congress for Electron Microscopy, Philadelphia, 1962). Ed.: S.S. Breese, Academic Press, New York, N.Y., p. P 2, 1962.

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MacKenzie, A.P. and Luyet, B.J.: An electron microscope study of the fine structures of very rapidly frozen blood plasma. Biodynamica 9:147-164, 1963.

MacKenzie, A.P. and Luyet, B.J.: Apparatus for freeze-drying at very low, controlled temperatures. Biodynamica 9:177-191, 1964.

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MacKenzie, A.P. and Luyet, B.J.: Effect of recrystallization upon the velocity of freeze- drying. In "Progress in Refrigeration Science and Technology" (Proceedings of the XIth. International Congress of Refrigeration, Munich, 1963). Pergamon Press, New York, N.Y. VI C-2, pp. 1573-1581, 1965.

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Arenberg, I.K., Marovitz, W.F. and MacKenzie, A.P.: Preparative techniques for the study of soft biological tissues in the scanning electron microscope: A comparison of air drying, low temperature evaporation and freeze-drying. In Proceedings of the Third Annual Stereoscan Colloquium." Kent Cambridge Scientific, Inc., Morton Grove, Ill., pp. 121-143, 1970.

George, R.P., Albrecht, R.M., Raper, K.B., Sachs, I.B. and MacKenzie, A.P.: Rapid freeze preparation of Dictyostelium discoideum for scanning electron nicroscopy. In Proceedings of the Third Annual Stereoscan Colloquium. Kent Cambridge Scientific, Inc., Morton Grove, Ill., pp. 159-165, 1970.

Rasmussen, D.H. and MacKenzie, A.P.: The glass transition in amorphous water. Application of the measurements to problems arising in cryobiology. J. Phys. Chem. 75:967-973, 1971.

Arenberg, I.K., Marovitz, W.F. and MacKenzie, A.P.: Preparative techniques for the study of soft biologic tissues in the scanning electron microscope. Trans. Amer. Acad. Ophthalmology and Otolaryngology, 75:1333-1345, 1971.

Albrecht, R.M., Hinsdill, R.D., Sandok, P.L., MacKenzie, A.P. and Sachs, I.B.: A comparative study of the surface morphology of stimulated and unstimulated macrophages, prepared without chemical fixation for scanning electron microscopy. Exptl. Cell Res. 70:230-232, 1971.

MacKenzie, A.P.: Freezing, freeze-drying, and freeze-substitution. In "Scanning electron microscopy/1972. Ed.: 0. Johari, Illinois Institute of Technology, Chicago, Illinois. pp. 274-280, 1972.

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Albrecht, R.M., Orndorff, G.R. and MacKenzie, A.P.: Survival of certain microorganisms subjected to rapid and very rapid freezing on membrane filters. Cryobiology 10:233-239, 1973.

Orndorff, G.R. and MacKenzie, A.P.: The function of the suspending medium during the freeze-drying preservation of *Escherichia coli*. Cryobiology 10:475-487, 1973.

MacKenzie, A.P.: Collapse during freeze-drying — Qualitative and quantitative aspects. In "Freeze-Drying and Advanced Food Technology." Ed.: S.A. Goldblith, L. Rey and W.W. Rothmayr. Academic Press Ltd., London. Pp. 277-307, 1975.

Albrecht, R.M. and MacKenzie, A.P.: Tissue cultured and free living cells. In "Principles and Techniques of Scanning Electron Microscopy, Vol. 3." Ed.: M.A. Hayat, Van Nostrand Reinhold Co., N.Y. Pp.109-153, 1975.

MacKenzie, A.P.: The physico-chemical environment during the freezing and thawing of biological materials. In "Water Relations in Foods." Ed.: R.B. Duckworth, Academic Press, London. Pp. 477-503, 1975.

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MacKenzie, A.P.: Principles of freeze-drying. Transplantation Proc., No. 2 (Suppl. 1): 181-188, 1976.

MacKenzie, A.P.: Physico-chemical basis for the freeze-drying process. In: "Developments in Biological Standardization", Vol. 36. Ed.. V.J. Cabasso and R.H. Regamey, S. Karger, Basel. Pp. 51-67, 1977.

MacKenzie, A.P.: Comparative studies on the freeze-drying survival of various bacteria: Gram type, suspending medium, and freezing rate. In: "Developments in Biological Standardization", Vol. 36. Ed.: V.J. Cabasso and R.H. Regamey, S. Karger, Basel. Pp. 263-277, 1977.

MacKenzie, A.P.: Solvent exchange and removal - Lyophilization. In: "Proceedings of the NHLBI Workshop on Technology for Protein Separation and Improvement of Blood Plasma Fractionation." DHEW Publication No. (NIH) 78-1422, pp. 185—201, 1978.

MacKenzie, A.P.: Modeling the ultra-rapid freezing of cells and tissues. In: "Microprobe Analysis of Biological Systems." Ed.: T.E. Hutchinson and A.P. Somlyo. Academic Press, New York. Pp. 397-421, 1981.

MacKenzie, A.P.: Simultaneous DTA and determination of electrical resistance of aqueous solutions during freezing and thawing. In: Proceedings of the 17th. NATAS Conference. Ed.: C.M. Earnest. North American Thermal Analysis Society, Rome, GA, pp. 9-14, 1988.

MacKenzie, A.P.: Freeze-drying of aqueous solutions containing peptides and proteins. In: Therapeutic Peptides and Proteins: Formulation, Delivery and Targeting. Eds.: D.R. Marshak and D.T. Liu. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Pp. 17-21, 1989.

D. Nochlin, A. P. MacKenzie, E. M. Bryant, I. H. Norwood, and S. M. Sumi. 1993, A Simple Method of Rapid Freezing Adequately Preserves Brain Tissue for Immunocytochemistry, Light and Electron Microscopic Examination. Acta Neuropathologica, 86, 645-650.

D. M. Strong and A. P. MacKenzie.1993. Freeze-Drying of Tissues. In: Musculoskeletal Tissue Banking, W. W. Tomford, Editor, Raven Press, New York, Pp. 181-208.

K. R. Morris, S. A. Evans, A. P. MacKenzie, D. Scheule, and N. G. Lordi. 1994. Prediction of Lyophile Collapse Temperature by Dielectric Analysis. PDA Journal of Pharmaceutical Science & Technology, 48:318-329.

S. A. Evans, K. R. Morris, A. P. MacKenzie, and N. G. Lordi. 1995. Dielectric Characterization of Thermodynamic First Order Events in Model Frozen Systems Intended for Lyophilization. PDA Journal of Pharmaceutical Science & Technology, 49:2-8.

W.R. Gombotz, S. C. Pankey, L. S. Bouchard, D. H. Phan, and A. P. MacKenzie. 1996.Stability, Characterization, Formulation, and Delivery System Development for transforming Growth Factor-Beta. In: Formulation, Characterization, and Stability of Protein Drugs, R. Pearlman and Y. J. Wang, Editors, Plenum Press, New York, pp. 219-245.

The following is a brief, but accurate, synopsis of my relevant experience:

Dr. MacKenzie began his continuous career in freezing and freeze-drying in 1959. His earliest studies centered on the fundamental aspects of the freezing and freeze-drying processes. Later his work focused also on pharmaceutical, microbiological and diagnostic applications. Dr. MacKenzie has published more than 50 papers on lyophilization and presented more than 100 papers

from the platform at national and international meetings. Dr. MacKenzie has developed and currently maintains a practical and theoretical interest in the biophysics and hydration of peptides and proteins and their freezing and freeze-drying behavior.

I have read and understood the specification and claims of the subject application, the Office Action dated July 19, 2000, and the Office Action dated September 20, 2001;

and, being as duly qualified, do further declare:

I. The present invention is based on the discovery that Factor VIII compositions can be prepared in a stable dried form by freeze-drying an aqueous solution of Factor VIII using trehalose as a stabilising agent in the absence of albumin.

II. I have been asked to review an issued United States patent to determine its relevance to the present invention. Specifically, I have reviewed the following reference:

Livesey *et al.* (U.S. Patent No. 5,364,756)

III. I have carefully reviewed this reference and, for the reasons discussed below, conclude that there is no teaching or suggestion in this reference of the present invention.

IV. My review of this reference has been done in the context of what was known in the art when the present invention was made. My knowledge in this regard is based on many years of experience in this field dating back to 1959. In 1991, various Factor VIII compositions were known. Factor VIII preparations which were derived from human blood necessarily contained albumin. Although there were significant health risks associated with administering albumin to a patient, namely the potential risk for viral contaminants to be present, the presence of albumin was believed to be necessary in order to stabilise the Factor VIII protein. Recombinant Factor VIII preparations were also being prepared, but again it was believed to be necessary to add albumin to the preparations to stabilise the proteins. The

presence of albumin was believed to be necessary because Factor VIII proteins are extremely labile, even in the presence of other stabilisers.

V. The teachings of the cited reference are entirely consistent with what was generally believed in the art at that time — that albumin was necessary to stabilise Factor VIII compositions. Although the presence or absence of albumin in a Factor VIII composition was clearly not the main focus of this reference, a careful review of this reference reveals evidence which supports the proposition that one skilled in the art would not have expected to be able to freeze dry Factor VIII in the absence of albumin.

VI. The Livesey *et al.* patent describes the cryopreservation of biological materials. The method and apparatus described by Livesey *et al.* can purportedly be used for cryopreservation of materials ranging from viruses to cultured mammalian cells. However, it is apparent from the disclosure, including the examples, that the primary focus is on the preservation of whole cells. Red blood cells, plateletes, leukocytes, sperm, pancreatic islets, and marrow cells are all listed as specific examples of cells which can be preserved using the Livesey *et al.* procedures. There is very little discussion, and there are no examples, of the preservation of proteins. Those skilled in the art know that materials and procedures used to preserve whole cells and/or viruses are not necessarily applicable to the stabilisation of proteins. Furthermore, Livesey *et al.* state that the exact ingredients of the suspensions which are to be preserved "is not considered to be a component of the invention." Thus, I find no disclosure in the Livesey *et al.* patent which is specifically relevant to the selection of appropriate stabilising agents for delicate proteins.

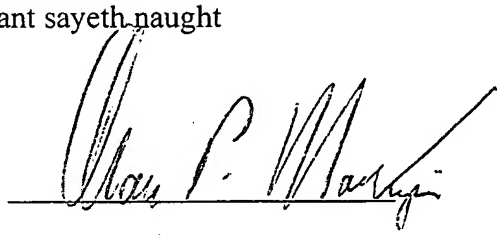
VII. Freeze drying of biological materials is a very complex, and poorly understood, process. Livesey *et al's.* extensive discussion of this process contains much information which is not relevant to the current review. However, the critical differences between ambient drying and cold temperature drying are expressly acknowledged by Liversey *et al.* It

is clear from the discussion in the Livesey *et al.* reference that protection of proteins from damage due to freezing involves very different mechanisms from protecting proteins from damage due to drying. Livesey *et al.* contrast the role of cryoprotectants with dry protectants stating that "[t]he cryosolution may also include exposing the biological suspension to one or more dry protectant compounds. Dry protectants, by definition, stabilize samples in the dry state." Livesey *et al.* specifically list human serum albumin as a cryoprotectant which has been found to be effective in combination with trehalose (a dry protectant). Thus, the Livesey *et al.* reference does not teach that trehalose can be used in the absence of albumin.

VIII. Trehalose is only mentioned by Livesey *et al.* as one possible ingredient in a drying process. There is no reference to preparing a Factor VIII composition in the absence of albumin, and it cannot be inferred, given the knowledge at that time, that albumin was not required. Thus, the Livesey *et al.* discussion of stabilising agents is entirely consistent with the proposition that Factor VIII cannot be freeze-dried without the use of albumin as a stabilising agent. In my opinion, there is nothing in the Livesey *et al.* specification or claims which teaches, or even suggests, to those skilled in the art, that Factor VIII can be freeze-dried in the absence of albumin.

The undersigned declares further that all statements made herein of his own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the Application or any Patent issuing thereon.

Further declarant sayeth naught

Signed: 

Date: 10/18/01